

Amendments to the Claims

This listing of claims will replace all prior versions and listings of claims in the Application.

Listing of Claims

1. (Currently Amended) A method for identifying an agent which alters the interaction between a protein tyrosine phosphatase and a tyrosine phosphorylated polypeptide which is a substrate of the protein tyrosine phosphatase, comprising:

(a) contacting in a solution in the absence and in the presence of a candidate agent, a substrate trapping mutant of a protein tyrosine phosphatase and a detectably labeled tyrosine phosphorylated peptide which is a substrate of the protein tyrosine phosphatase under conditions and for a time sufficient to permit formation of a complex between the tyrosine phosphorylated peptide and the substrate trapping mutant protein tyrosine phosphatase, wherein the substrate is capable of generating a fluorescence polarization signal and wherein the substrate trapping mutant protein tyrosine phosphatase is selected from the group consisting of

(i) a protein tyrosine phosphatase in which wildtype protein tyrosine phosphatase catalytic domain invariant aspartate residue is replaced with an amino acid which does not cause significant alteration of the Km of the enzyme but which results in a reduction in Kcat to less than 1 per minute, and

(ii) a protein tyrosine phosphatase in which a cysteine that is present in a signature sequence motif as set forth in SEQ ID NO:1 within a wildtype protein tyrosine phosphatase catalytic domain is mutated; and

(b) comparing in the solution, without separating the complex from free substrate, the fluorescence polarization signal level in the absence of the agent to the fluorescence polarization signal level in the presence of the agent, wherein a difference in the fluorescence polarization signal level indicates the agent alters formation of a complex between the protein tyrosine phosphatase and the substrate.

2. (Cancelled)

3. (Original): The method of claim 1 wherein the detectably labeled tyrosine phosphorylated peptide comprises a fluorophore.

4. (Original): The method of claim 3 wherein the fluorophore is selected from the group consisting of fluorescein, rhodamine, Texas Red, AlexaFluor-594, AlexaFluor-488, Oregon Green, BODIPY-FL and Cy-5.

5. (Original): The method of claim 1 wherein the substrate comprises a polypeptide sequence derived from a protein selected from the group consisting of VCP, p130^{cas}, EGF receptor, p210 bcr:abl, MAP kinase, Shc, insulin receptor, lck and T cell receptor zeta chain.

6. – 7. (Cancelled)

8. (Previously presented): The method of claim 1 wherein the substrate trapping mutant protein tyrosine phosphatase comprises a protein tyrosine phosphatase in which at least one wildtype tyrosine residue is replaced with an amino acid that is not capable of being phosphorylated.

9. (Original): The method of claim 8 wherein at least one wildtype tyrosine residue is replaced with an amino acid selected from the group consisting of alanine, cysteine, aspartic acid, glutamine, glutamic acid, phenylalanine, glycine, histidine, isoleucine, lysine, leucine, methionine, asparagine, proline, arginine, valine and tryptophan.

10. (Original): The method of claim 8 wherein at least one tyrosine residue that is located in a protein tyrosine phosphatase catalytic domain is replaced.

11. (Original): The method of claim 8 wherein at least one tyrosine residue that is located in a protein tyrosine phosphatase active site is replaced.

12. (Original): The method of claim 8 wherein the wildtype tyrosine residue is replaced with phenylalanine.

13. (Original): The method of claim 8 wherein the wildtype tyrosine residue that is replaced is a protein tyrosine phosphatase conserved residue.

14. (Withdrawn): The method of claim 13 wherein the conserved residue corresponds to tyrosine at amino acid position 676 in human PTPH1.

15. (Original): The method of claim 8 wherein at least one tyrosine residue is replaced with an amino acid that stabilizes a complex comprising the protein tyrosine phosphatase and at least one substrate molecule.

16. (Original): The method of claim 8 wherein the substrate trapping mutant protein tyrosine phosphatase is a mutated protein tyrosine phosphatase selected from the group consisting of PTP1B, PTP-PEST, PTP γ , MKP-1, DEP-1, PTP μ , PTPX1, PTPX10, SHP2, PTP-PEZ, PTP-MEG1, LC-PTP, TC-PTP, CD45, LAR and PTPH1.

17. – 33. (Cancelled)